pE2 Conformational Antigenic Determinants Important in HEV Infection

As shown and described above, of the two types of pE2 antigenic activity recognizable by HEV reactive human sera, the conformational antigenic determinants of the dimeric form appear to play a more significant role in natural HEV infection than the linear epitopes of the monomeric form. This was concluded when the results of a Western blot analysis (Figure 10) showed that out of a total of 18 HEV reactive human sera, all 18 sera were reactive against the dimeric form of the pE2 peptide, while only 8 of the sera were additionally reactive against the monomeric form of the pE2 peptide. Furthermore, based on the results shown in Figure 9, the overall reactivity of the pE2 conformational antigenic determinants was estimated to be about 80 greater than that of the linear epitopes contained within the monomer.

The role of the pE2 conformational antigenic determinants in natural HEV infection was further evaluated by testing sera from 21 healthy blood donors and 96 patients having non-A, B and C acute hepatitis taken at different times after onset of hepatitis (Table 2). The sera used in the study was obtained from patients admitted to the Princess Margaret Hospital in Hong Kong with current, or a past history of non-A, B and C acute hepatitis. The results show that pE2 specific IgG antibody was markedly more prevalent among the non-A, B and C hepatitis patients than the healthy donors. The difference is consistent with epidemiological studies indicating that HEV is a common cause of non-A, B and C hepatitis. The detection of the IgG antibody suggests that some of these patients may be currently infected and others may have been previously infected with the virus. About 10% of the healthy donors had been infected with the virus in the past and this is in agreement with the level of HEV infection in the community from which these blood samples were taken.

Table 6
Determination of low avidity pE2 IgG antibody by ELISA in serum samples of non-A, B and C hepatitis patients at different times after onset of the disease.

	pE2 IgG Avidity Test (Cases)		
Days After Onset	Low Avidity	High Avidity	Total
0-7	3	0	3
8-14	3	3	6
15-21	3	0	3
22-84	0	12	12
>84	0	7	7
Total	9	22	31

VII Establishment of Immune Capture RT-PCR to Detect HEV RNA

The above described reactivity of human sera may be explained if the pE2 dimer were to mimic certain structural features of the HEV capsid. To test this possibility, experiments were carried out to determine if antisera raised against pE2 may effect immune capture of HEV particles.

Specificity of RT-PCR Primers

In this study, two pairs of primers, A5R/A3F and B5R/B3F (Table 9) were chosen for RT-PCR. The specificity of these primers was evaluated by direct RT-PCR with specimens separately containing HEV, HAV, enteroviruses and caliciviruses. Figure 16 shows that only the specimen containing HEV presented a specific band with the expected size of the 203 bp HEV sequence following RT-PCR amplification.

pE2 Dimer Mimics Structural Properties of HEV

The antigenic relationship between pE2 dimer and HEV viral particles was studied by immune capture experiments using polystyrene paddles coated with antisera raised against the pE2 peptide. The antiserum used was obtained from a monkey immunized with 4 doses of 100 μg of purified pE2 and which was predominantly reactive against the pE2 dimer. The

Table 7
Comparative Efficiency of HEV Detection by Immune Capture-RT-PCR and Conventional RT-PCR Methods

Method	Water	Stool Supernatant	Shellfish Supernatant
IC-RT-PCR	1:3,125	1:125	1:125
Conventional RT-PCR	1:125	1 (not diluted)	not detected

Sensitivity of detection is indicated as the limiting sample dilution required for virus detection.

In conclusion, both the commercial RT-PCR method and the IC-RT-PCR method have a lower sensitivity for the stool and shellfish samples than for the water sample. However, this phenomenon may be caused by some unknown factors in the stool and shellfish samples which can possibly accelerate the degradation of viruses. On the other hand, the IC-RT-PCR results are 125-fold more sensitive than using direct RT-PCR which implies that the immune capture method successfully overcame the interference from unknown facts in the stool and shellfish samples and further illustrates that IC-RT-PCR is practical as a clinical and environmental monitor.

VIII The Role of pE2 in HEV Protection

The previous results of the study of non-A, B and C hepatitis patients, indicated that the dimeric form of the recombinant peptide, pE2, may assume an important role in natural HEV infection through the exposure of conformational antigenic determinants which are generated from the dimerization of the monomeric form of the peptide.

As previously shown in the study of non-A to C acute hepatitis patients, the dimeric form of pE2 was found to assume a more prominent role in natural HEV infection. pE2 specific IgM antibodies were commonly produced during acute HEV infection. The corresponding IgG antibodies were also produced and persisted for a protracted period of time accompanied by increasing avidity. Furthermore, they were the most prevalent HEV antibodies present in convalescent sera and sera from individuals previously infected with the virus. These results suggested that pE2 may afford protection against HEV and is further supported by a protection study in the experimental infection of Macaque monkeys. Moreover, the results also suggest that the protective effects are mainly attributed to the conformational antigenic

Table 8
HEV Excretion in Stool and Viraemia After Virus Challenge

Group	Monkey	Day After Challenge 3	5	7	9	11	13	15	17	19	21
Test	No. 1	-	-	-	-	-	-	-	-	-	-
	No. 2	-	-	-	-	-	-	-	-	-	-
	No. 3	-	+	-	-	-	-	-	-	-	-
Control	No. 5	-	+	+	+	+	+[1]	+	+	-	-
	No. 7	-	-	+	+[1]	+	+	+	+	-	-
	No. 8	-	-	+	+	+	+	+	+	-	-

[1] The HEV genome was detected in peripheral blood monocytes by RT-PCR. None of the plasma samples contained detectable HEV genome.

HEV Seroconversion After Virus Challenge

The spectrum of HEV antibodies present in the serum samples obtained before and up to 4 weeks after virus challenge was analyzed according to the experimental methods and conditions described for Figure 19. Figure 21 shows that the sera obtained from all three control animals (M5,M7,M8) before virus challenge gave a negative result by all three HEV antibody assays initially. HEV seroconversion occurred 7 days following infection in one control animal, M5. This was evidenced by the detection of the pE2 homodimer specific antibody in sera taken on this day and all subsequent occasions (Figure 21). All these serum samples also gave a possible test result for HEV antibody by the commercial ELISA test and the consecutive samples obtained on the 7th day and 14th day were reactive against purified GE3 as well. The broad specificity spectrum of the antibody response to the infection was in contrast to the restricted specificity spectrum of the antibody response to immunization with the pE2 peptide described earlier (Figure 21). The serum specimens obtained from another control animal, M7, on the 14th and 21st days after infection were weakly reactive against the pE2 dimer and gave a positive result by the commercial assay, but none of the specimens were reactive against the ORF3 specified peptide, GE3. The remaining control animal, M8, did not mount a detectable antibody response to the infection. In contrast, except for the pE2 antibody, the immunized animals did not acquire other HEV antibodies after the infection (Figure 21).

The sera obtained from the immunized animals (M1,M2,M3) were already reactive against the pE2 dimer before the virus challenge on day 0 and the antibodies persisted in the